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(54) Method and composition for making liposomes.

(57) Mixtures of a hydrating agent such as arginine or glutamic acid and liposome-forming materials provide a pre-liposome gel which spontaneously forms highly stable liposomes in aqueous solution having very high capture efficiency.

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METHOD AND COMPOSITIONS FOR MAKING LIPOSOMES10 Background

This invention relates to novel liposome-forming compositions which provide a new method for forming liposomes. More specifically, this invention relates to the use of hydrating agents, compounds with at least two
15 ionizable groups, with liposome-forming materials to make a gel which spontaneously forms liposomes when diluted with an aqueous solution.

General Description

20 Numerous processes and methods have been developed for making the different types and sizes of liposomes and for encapsulating active ingredient. Most of these methods have focused on the use of an organic solvent to ensure complete solubilization and uniform mixing of the
25 phospholipids and fatty acids prior to dispersion in an aqueous system. A second development was the use of ultrasonic irradiation to disperse the phospholipid/fatty acid material.

For example Robinson, Trans. Faraday Soc., 56:1260
30 (1960) and Papahadjopoulos, et al. [Biochim. Biophys. Acta, 135, 639 (1967)] describe formation of phospholipid dispersions from a two-phase ether/water system involving evaporation of the ether by bubbling nitrogen through the mixture. Similarly, chloroform has
35 been used by Chowhan, et al., Biochim. Biophys. Acta, 266:320 (1972) to insure a complete and thorough mixing of

16501

1 the phospholipids prior to dispersion.

Ultrasonic dispersion, first described by D. Papahadjopoulos and N. Miller in Biochim. Biophys. Acta, 135:624 (1967) produces small unilamellar vesicles but the
5 technique is limited because of low encapsulation efficiency.

Batzri and Korn [Biochim. Biophys. Acta, 198:1015 (1973)] have used the technique of injecting the lipids in an organic phase (ethanol) into an aqueous solution.
10 Ether was used by Deamer and Bangham in essentially the same technique [Biochim. Biophys. Acta, 443:619 (1976)].

Yet another technique involves a calcium-induced structural change in a lipid vesicle derived from or
15 containing phosphatidylserine but is reported to have a relatively low encapsulation efficiency due to the method of reconstitution of the vesicle. See Papahadjopoulos, et al., Biochim. Biophys. Acta, 394:483 (1975) and H. Hauser, Trends in Pharm., Science 3, 274-77 (1982).

20 Several other patents set out methods for lipid vesicle formation of interest to this invention. U.S. patent No. 3,804,776 describes a method for producing oil and fat encapsulated amino acids for polypeptides by dispersing dry powders of the material in a molten mixture
25 of the fat or oil and then pouring the mixture into water. The encapsulated material is contained within relatively large droplets of lipid. Such vesicles are not suitable for IV injection and are limited to use only for oral administration.

30 Entrapment of certain drugs in lipid vesicles by freezing the aqueous phospholipid dispersion of the drug and lipid is described in U.S. patent No. 4,016,100.

Papahadjopoulos and Szoka, in U.S. patent No. 4,235,871, disclose a method for making liposomes where
35 the vesicle-forming material is dissolved in an organic solvent and then mixed with an aqueous solution containing

1 the material to be encapsulated. An homogeneous
water-in-oil emulsion is formed and the organic solvent is
evaporated to give a gel-like mixture. This gel is then
suspended in water to form the vesicles.

5 Another process of interest is disclosed in U.S.
patent No. 3,932,657, which teaches the encapsulation of
polyaminopolycarboxylic chelating agents, EDTA and TDPA.
Yet another U.S. patent, 4,217,344, issued to
Vanlerberghe, et al., notes that certain additives can be
10 combined with nonionic lipid compounds so as to modify the
permeability and superficial charge of lipid vesicles.
Among the several additives mentioned are polypeptides and
proteins. Mackaness, et al. describe in U.S. Patent
4,192,859, contrasts media containing liposomes as
15 carriers. The list of contrast agent salts includes
arginine salt, cystein salt, glycine salt, glycyI glycine
salt, and N-methylglucosamine salts. These materials are
characterized as aliphatic and alicyclic amines which can
be used to prepare water soluble salts of the various
20 contrast agents which may be employed in X-ray contrast
agents.

An article in Science, March, 1984, by Janos Fendler
makes reference to a number of synthetic surfactants which
may be used in forming vesicles. Fendler references
25 quaternary ammonium and carboxylate, sulfate, sulfonate
and phosphate zwitterionic materials which are referenced
to the following literature articles: J. H. Fendler, Acc.
Chem. Res., 13, 7 (1980); T. Kunitake and S. Shinkai, Adv.
Phys. Org. Chem., 17, 435 (1980); T. Kunitake, et. al., J.
30 Am. Chem. Soc., 103, 5401 (1981); J. H. Fuhrhop and J.
Matthieu, J. Chem. Soc. Chem. Commun. p. 141 (1983); and
W. Talmon, et. al., Science, 221, 1047 (1983).

It has been discovered that liposomes are
spontaneously formed when phospholipids and/or fatty
35 acids, liposome forming materials, are dispersed in an
aqueous medium containing a hydrating agent. This class

1 of compounds, for the purpose of this invention, is
illustrated by arginine and similar amino acids which have
at least one ionizable functionality at both the alpha and
omega termini of the molecule. These "hydrating"
5 compounds will have either the same type of ionizable
group on the molecule, both cationic, or both anionic, or
have ionizable groups of opposite charge. It isn't
required that the ionizable groups be on the alpha and
omega carbons but such compounds represent the preferred
10 embodiments of this invention.

In practical terms, liposomes formed using this
invention are formulated as a "pre-liposome gel" referred
to herein as a "gel" where a phospholipid and/or fatty
acid mixture capable of forming liposomes is mixed with an
15 appropriate, concentrated aqueous solution of the
hydrating compound. This gel, upon dispersion in an
aqueous medium efficiently and spontaneously forms
liposomes without solvent evaporation, input of ultrasonic
irradiation or any of the other means developed to insure
20 proper formation of lipid vesicles, liposomes.

Liposomes made with hydrating agents are more stable
than the ones produced by conventional methods, including
those formed using organic solvents and ultrasonic
energy. Liposome formulations having these hydrating
25 agents suffer none of the solvent removal problems of the
current technology nor are the liposomes beset by the
non-uniform, destructive forces of ultrasonic irradiation
inherent in the older methods.

Additionally, the pre-liposome gel can be dehydrated
30 and stored for a substantial period of time and still be
capable of spontaneously forming liposomes upon
rehydration.

The pre-liposome gel is extraordinarily stable,
stable enough to be autoclaved for sterilization.
35 Furthermore, water-soluble or water-insoluble substances
to be encapsulated can be added to the gel and will then

1 be incorporated into the liposomes upon dispersion of the
gel. This capability has the effect of greatly enhancing
the encapsulation efficiency.

Furthermore, it also has been discovered that the
5 concentration of hydrating agent influences the size of
the resulting liposomes in a predictable manner at a given
pH. Correspondingly, varying the pH of the dispersing
aqueous solution while holding the hydrating agent
constant also influences the size of the liposomes
10 produced in a predictable manner.

Thus, the present invention provides an easier, more
convenient and predictable means for controlling vesicle
size over methods previously available. This method also
has no limitations on the concentrations of lipids in the
15 preparation of liposomes.

Summary of the Invention

This invention relates to a liposome product made by
dispersing in an aqueous medium, in a manner adequate to
20 form liposomes, a composition comprised of:

- a. liposome-forming material;
- b. a hydrating agent wherein the hydrating agent is
present in a molar ratio of between 1:20 and 1:0.05
relative to the liposome-forming material;
- 25 c. water in an amount up to 300 moles relative to the
solids; and
- d. optionally, a material to be encapsulated.

In a second aspect, this invention covers a
composition capable of forming liposomes when dispersed in
30 an aqueous medium, which composition comprises:

- a. liposome-forming material;
- b. a hydrating agent wherein the hydrating agent is
present in a molar ratio of between 1:20 and 1:0.05
relative to the liposome-forming material;
- 35 c. optional material to be encapsulated; and
- d. water in an amount up to 300 moles relative to the

1 solids.

In another aspect, this invention relates to a means for forming stable liposomes wherein the means comprises adding a hydrating agent to liposome-forming materials in
5 a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material and dispersing the mixture in an aqueous medium in a manner adequate to form liposomes. Alternatively, the hydrating agent, liposome-forming material and the substance to be encapsulated can be added
10 separately to the aqueous medium, then means for dispersion applied to form the liposomes.

SPECIFIC EMBODIMENTS

15 Definitions

For the purpose of this invention, a hydrating agent means a compound having at least two ionizable groups, preferably of opposite charge, one of which is capable of forming an easily dissociative ionic salt, which salt can
20 complex with the ionic functionality of the liposome-forming material. The hydrating agent inherently does not form liposomes in and of itself. Such agent will also be physiologically acceptable, i.e., it will not have any untoward or deleterious physiological effect on the
25 host to which it is administered in the context of its use in this invention.

Complexing in this context denotes the formation of dissociative ionic salts where one functionality associates with the ionic functionality of the
30 liposome-forming material and the other functionality has hydrophilic properties which impart water-solubility to the resulting complex.

Hydrated complex means the complex formed between the hydrating agent and the liposome-forming material whereby
35 there is formed a specific semi-crystalline arrangement of molecules. Certain particular, specific spectral data

1 characterized the presence of this complex.

Mixtures of the hydrating agent and liposome-forming materials with certain, discrete amounts of water form a gel-like mass. When in this gel form, the hydrating agent and the liposome-forming material arrange into a "hydrated complex" which is a highly ordered liquid crystal. While the liquid crystal structure varies with pH and amount of hydrating agent, the liquid crystal structure remains. NMR spectroscopy confirms that the crystal structure consists of multilamellar lipid bilayers and hydrophilic layers stacked together in alternating fashion. The ³¹P-NMR spectrum exhibits an anisotropic peak, further confirming the existence of multilamellar bilayers.

The word "liposome" has been proposed and accepted as the term to be used in the scientific literature to describe synthetic, oligolamellar lipid vesicles. Such vesicles are usually comprised of one or more natural or synthetic lipid bilayers surrounding an internal aqueous phase.

20 The phrase "liposome-forming material" refers to all natural and synthetic compounds which have one ionizable function and a hydrophobic component, a fatty component, such as the phospholipids, non-volatile fatty acids, non-volatile alkyl amines and the like which singly or in combination form liposomes when dispersed in an aqueous medium. This definition is not intended to be limiting in its scope but is to be read to include all compounds capable of forming lipid vesicles, past, present and future.

30 Examples of liposome-forming materials include saponifiable and non-saponifiable lipids, e.g., the acyl glycerols, the phosphoglycerides, the sphingolipids, the glycolipids, etc. The fatty acids include saturated or unsaturated alkyl (C₈-C₂₄) carboxylic acids, 35 mono-alkyl (C₈-C₂₇) esters of C₄-C₁₀ dicarboxylic acids (e.g., cholesterol hemi-succinic acid

- 1 and fatty acid derivatives of amino acids in which any
 N-acyl carboxylic acids also are included (e.g., N-oleoyl
 threonine, N-linoleoyl serine, etc.). Mono- or di-alkyl
 (C_8-C_{24}) sulfonate esters and mono- or di-alkyl
 5 (C_8-C_{24}) phosphate esters can be substituted for the
 fatty acids. Furthermore, mono- or di-acyl (C_8-C_{24})
 glycerol derivatives of phosphoric acids and mono- or
 di-acyl (C_8-C_{24}) glycerol derivatives of sulfuric
 acids can be used in place of the fatty acids.
- 10 Additionally, the fatty acids also can be replaced by
 saturated or unsaturated alkyl amines (e.g., C_8-C_{24}
 NH_2), C_8-C_{24} fatty acid derivatives of amines
 (e.g., $C_8-C_{24} CONH-NH_2$), C_8-C_{24} fatty
 alcohol derivatives of amino acids (e.g., C_8-C_{24}
 15 $OOC-NH_2$), and C_8-C_{24} fatty acid esters of amines
 (e.g., $C_8-C_{24} COO-NH_2$).

Photopolymerizable lipids and/or fatty acids (or
 amines) (e.g., diacetylenic fatty acids) also can be
 included, which can provide a sealed liposome with
 20 cross-linked membrane bilayers upon photo-initiation of
 polymerization.

Although the primary components of these liposomes
 will be lipids, phospholipids, other fatty acids, there
 may also be added various other components to modify the
 25 liposomes' permeability. There may be added, for example,
 non-ionic lipid components such as polyoxy alcohol
 compounds, polyglycerol compounds or esters of polyols,
 polyoxyalcolinolated alcohols; the esters of polyols and
 synthetic lipolipids, such as cerebrosides. Other
 30 materials, such as long chain alcohols and diols, sterols,
 long chain amines and their quaternary ammonium
 derivatives; polyoxyethylenated fatty amines, esters of
 long chain amino alcohols and their salts and quaternary
 ammonium derivatives; phosphoric esters of fatty alcohols,
 35 polypeptides and proteins.

The composition of the liposome can be made of more

1 than one component of the various kinds of lipids, the
fatty acids, alkyl amines, or the like, and the hydrating
agents.

It also has been discovered that the lipid
5 composition may not require the inclusion of the fatty
acids (or the amines) or the hydrating agents to form the
"pre-liposome gel" or liposomes, if the lipid component
itself or the substances (e.g., medicaments, biologically
active compounds, cosmetics, etc.) to be encapsulated
10 possess the aforementioned properties. For example, the
mixture of dipalmitoylphosphatidylcholine (DPPC) and
distearoyl phosphatidylethanolamine forms the
"pre-liposome gel" or liposomes with aqueous glutamic acid
solution and the mixture of DPPC and oleic acid with
15 aqueous epinephrine solution forms the "pre-liposome gel"
and liposomes.

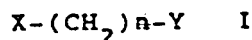
For pharmaceutical application as a liposome drug
delivery system, however, the composition of
phospholipids, oleic acid (or phosphatidylethanolamine)
20 and arginine or lysine (or glutamic acid and/or aspartic
acid) was preferred.

When solids are referred to, the liposome-forming
materials, hydrating agents, and material to be
encapsulated if any, is what is being referred to.

25

Preferred Embodiments

The preferred hydrating agents of this invention are
alpha amino acids having an ionizable omega substitution
such as a carboxylate, amino, and guanidino function and
30 those compounds represented by the formula:



wherein

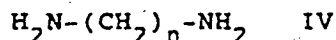
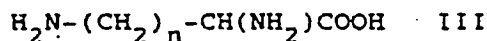
35 X is $H_2N-C(NH)-NH-$, H_2N- , ZO_3S- , Z_2O_3P- , or
 ZO_2C- wherein Z is H or an inorganic or organic cation;

1 Y is $-\text{CH}(\text{NH}_2)-\text{CO}_2\text{H}$, $-\text{NH}_2$, $-\text{NH}-\text{C}(\text{NH})-\text{NH}_2$, $-\text{COOH}$,
 $\text{CH}(\text{NH}_2)\text{SO}_3\text{Z}$ or $\text{ZH}(\text{NH}_2)\text{PO}_3\text{Z}_2$ wherein Z is defined
 above; and

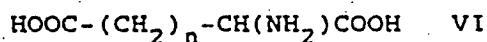
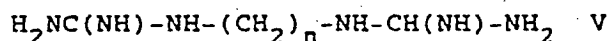
n is the integer 1-10; or

5 a pharmaceutically acceptable salt thereof. Also
 included in the list of preferred compounds are the
 $\text{N,N}'$ -dialkyl substituted arginine compounds and similar
 compounds where the alkyl chain length is varied.

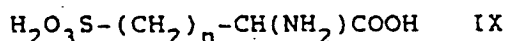
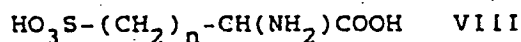
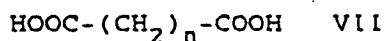
More preferred hydrating agents are the
 10 omega-substituted, alpha amino acids such as arginine, its
 N-acyl derivatives, homoarginine, gamma-aminobutyric acid,
 asparagine, lysine, ornithine, glutamic acid, aspartic
 acid or a compound represented by the following formulae:



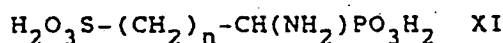
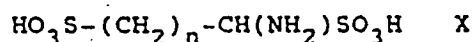
20



25



30



35 wherein n is 2-4.

The most preferred compounds are arginine,

1 homoarginine, gamma-aminobutyric acid, lysine, ornithine,
glutamic acid or aspartic acid.

About 1:20 molar ratio of hydrating agent relative to
the liposome-forming material will provide the salutary
5 effects of this invention with an upper limit of about
1:0.05. The preferred concentration range for the
hydrating agent is between a 1:2 to 1:0.5 molar ratio of
the hydrating relative to the liposome-forming material.

The hydrating agents of this invention may be used
10 alone or as a mixture. No limitation is implied or
intended in the use of mixtures of these hydrating
materials.

As a practical matter, thus a matter of preference,
if liposomes are prepared with liposome-forming materials
15 having a negative charge, it is preferred to use hydrating
agents which contain at least one ionizable nitrogen, such
as arginine, homoarginine, lysine, ornithine, and the
like. Conversely, if the amphipatic materials used to
form the liposomes are nitrogen-based, it is preferred to
20 use a di-acid such as glutamic acid, aspartic acid; any of
the alkyl di-acids such as the simple di-acids such as
valeric acid, caprylic, caproic, capric or the like; or
those di-acids having two phosphate, or sulfate
functionalities; or those di-acids having mixed
25 -COOH/-SO₃H or -COOH/-PO₃H₂ functions.

Source of hydrating agents

The hydrating agents of this invention are listed in
the catalogue of many chemical producers, can be custom
30 manufactured by such producers, or can be made by means
known in the art.

Arginine, homoarginine, lysine, glutamic acid,
aspartic acid, and other naturally occurring amino acids
may be obtained by the hydrolysis of protein and
35 separation of the individual amino acids or from bacterial
sources.

1 The compounds of formula II can be made by the method
of Eisele, K. et al, Justusliebig's. Ann. Chem., p 2033
(1975). Further information on several representative
examples of these compounds is available through their
5 respective Chemical Abstracts Service numbers as follows:
norarginine, CAS # 14191-90-3; arginine, CAS # 74-79-3;
and homoarginine, CAS # 151-86-5.

For representative examples of formula III, see for
2,4-diaminobutanoic acid CAS # 305-62-4 and for lysine
10 CAS # 56-87-1.

Methods for making representative compounds of
formula IV are available from Chemical Abstracts as
follows: ethane diamine, CAS # 305-62-4; propane diamine -
54618-94-9; and 1,4-diaminobutane, CAS # 52165-57-8. See
15 specifically Johnson, T.B., J. Am. Chem. Soc., 38, 1854
(1916).

Of the compounds of formula VI, glutamic acid is well
known in the art and is available from many commercial
sources. How to make other representative compounds is
20 contained in the literature, for example:
2-aminohexandioic acid - CAS # 62787-49-9 and
2-aminoheptandioic acid - CAS # 32224-51-0.

Glutamic acid, the compound of formula VII where n is
2 is well known in the art and can be made by the method
25 of Maryel and Tuley, Org. Syn., 5, 69 (1925). Other
representative compounds in this group can be made
according to the art as referenced by the following CAS
numbers: hexadioic acid, CAS # 123-04-9 and heptadioic
acid, CAS # 111-16-0.

30 Homocysteic acid is known in the art referenced by
CAS # 56892-03-6. The compound 3-sulfovaline is described
in the literature referenced by CAS # 23405-34-2.

Pre-liposome Gel

35 Mixtures of liposome-forming materials and one or
more hydrating agents with up to 300 moles of water

1 relative to the total liposome-forming material gives a
gel which forms liposomes directly therefrom upon addition
of an aqueous medium. This gel is labeled a
pre-liposome gel because i.) of its structural
5 characteristics which are essentially those of liposomes
and, ii.) the gel's facility for being converted into
liposomes upon dilution with an aqueous medium. Aqueous
medium in excess of about 300 moles cause the beginning
of liposome formation.

10 The structure of this gel is a highly ordered liquid
crystal which forms an optically clear solution. The X,
Y, and Z dimensions of the liquid crystal vary with the
concentrations of hydrating agent at the constant pH as
well as with the pH of the solution. By varying the
15 hydrating agent concentration at constant pH or changing
the pH while maintaining percentage of hydrating agent,
the size and number of lamellae structures of the lipid
bilayers of the subsequent liposome vesicles can be
controlled.

20 The gel structure itself can accommodate up to
approximately 300 moles of water per mole of lipid or
fatty acid without disturbing the stability of the gel
structure. The structure of the gel as determined by
proton NMR spectroscopy is comprised of multilamellar
25 lipid bilayers and hydrophilic layers stacked together in
an alternating fashion. The ^{31}P -NMR spectrum of the
same gel exhibits an anisotropic peak further confirming
that the gel consists of a multilamellar bilayer.

This gel can be autoclaved, a convenient means of
30 sterilization. Furthermore, the gel shows no
discoloration and remains clear at room temperature for at
least one year after being autoclaved. The gel can
further be sterilized by filtration through an appropriate
sterilization filter.

35 Upon dispersion of the gel into an aqueous medium,
liposomes are efficiently and spontaneously produced.

1 The pre-liposome gel, with or without the material to
be encapsulated, also can be dehydrated (lyophilized) and
the powder rehydrated to form liposomes spontaneously,
even after a long period of storage. This capability
5 makes the invention particularly useful for administering
water-sensitive medicaments where long term pre-use
storage is needed.

Either water insoluble or water soluble chemicals,
drugs, cosmetics, food materials and the like, can be
10 incorporated into liposomes prepared using this material
and by this method. Accordingly, the gel may be used as a
delivery system for the administration of medicaments via
oral, parenteral, topical, intravenous, suppository routes
or any other route of drug or chemical delivery.

15 The use of these liposomes is not limited to human or
mammalian use but can be used in any industrial,
agricultural, pharmaceutical, cosmetic or chemical
application where lipid vesicle encapsulation and
administration of compounds or materials is warranted or
20 useful.

The versatility of the present invention is
illustrated, but not limited, by the following examples.

Example #1

25 Preparation of Liposomes (Gel Phase)

Dipalmitoylphosphatidylcholine, 3.0 grams, was
weighed into a 50 ml beaker. Oleic acid 1.2 grams was
added and mixed together to form a uniform paste.

Arginine 0.72 grams in 30 ml of distilled deionized
30 water was added to the
dipalmitoylphosphatidylcholine-oleic acid paste and heated
to 45°C. With mixing by hand, the mixture formed a
clear stable gel. The gel was stored and liposomes later
formed by diluting the gel with phosphate buffered saline.

35

1

Example #2Preparation of Liposomes

Dipalmitoylphosphatidylcholine, 120 mg, and 24 mg of oleic acid were added together and mixed thoroughly until
5 a white homogeneous paste was observed.

Then 20 mg of arginine was dissolved into 60 ml of phosphate buffered saline (ionic strength = 0.15, pH = 7.4). The arginine-saline solution was added to the paste and heated to 40°C for 1/2 hour, or until a slightly
10 turbid solution was observed.

Example #3Large Scale Gel and Liposome Preparation

i). Gel Manufacture: To 50 grams of egg phosphatide powder type 20 (Ashai Chemicals) was added 20 grams of
15 oleic acid N.F. Mixing gave a white paste which was cooled to 4°C and ground into a fine powder. This powder was added to an aqueous solution containing 20 grams of arginine and 500 grams of distilled deionized
20 water. The mixture was mixed with a spatula as the solution was heated to about 35°C to help hydrate phospholipids. A homogeneous, slightly yellow gel was formed. This gel can be stored at 4°C or can be frozen and later reconstituted.

25 ii). Manufacture of Liposomes: The gel prepared in the preceding paragraph was taken from cold storage and returned to room temperature. It was then mixed with 2 liters of phosphate buffered saline, pH 7.4. A white opaque liposome solution was formed.

30

Example #4Liposome Formation from the Gel

A homogeneous paste of 1.0 gram of dipalmitoylphosphatidylcholine (DPPC) and 400 mg of oleic
35 acid was formed. Then 300 mg of arginine was mixed in 10 ml of phosphate buffered saline, heated to 45°C and

1 added to the DPPC/oleic acid paste to form liposomes.

Example #5

Pre-Liposome Gel

5 One gram of dipalmitoylphosphatidylcholine (DPPL) was mixed with 400 mg of oleic acid to form a homogeneous paste. 300 mg of arginine was mixed with 2 ml of water at 45°C until dissolved. The arginine solution was mixed with the DPPC/oleic acid paste at about 45°C to give a
10 thick gel. Liposomes formed when this gel was diluted with phosphate buffered saline.

Example #6

Cholesterol Containing Liposomes

15 Cholesterol, 15 mg, was mixed with 100 mg dipalmitoylphosphatidylcholine (DPPC) to form a homogeneous powder. Then 23 mg of oleic acid was added to the powder and thoroughly mixed to form a homogeneous paste. To make liposomes, 30 mg of arginine was added to
20 10 ml of phosphate buffered saline, heated to 40°C and added to the DPPC/cholesterol/oleic acid paste. The combination was mixed at 40°C to obtain liposomes.

Example #7

25 Palmitic Acid-Containing Liposomes

Dipalmitoylphosphatidylcholine (DPPC) 250 mg was mixed with 25 mg of palmitic acid to form a uniform powder. Then 80 mg of oleic acid was mixed with this powder and heated to 45°C with constant stirring until a
30 uniform paste was formed. Arginine 100 mg was dissolved in 25 ml of distilled deionized water and heated to 45°C. This arginine solution was added to the paste at 45°C and mixed until a uniform homogeneous gel was formed. The gel was diluted ten fold with phosphate
35 buffered saline to form liposomes.

1

Example #8Isostearic Acid-Containing Liposomes

Dipalmitoylphosphatidylcholine 100 mg was mixed with 50 mg of isostearic acid to form a uniform homogeneous paste. An arginine solution of 50 mg of arginine in 2.0 ml of distilled deionized water was made and added to the isostearic acid paste and heated to 45°C. The mixture was mixed until a clear gel was formed. Liposomes are formed upon dilution with phosphate-buffered saline.

10

Example #9Oleoyl Threonine Containing Liposomes

Dipalmitoylphosphatidylcholine 125 mg and 75 mg of oleoyl threonine were added together and heated to 40°C to form a paste. Then 2 ml of distilled deionized water was added with constant mixing at 40°C. A clear gel was formed which can be diluted with phosphate buffer saline at pH 5 to form liposomes.

20

Example #10Myristyl Amine Containing Liposomes

Dipalmitoylphosphatidylcholine 192 mg was added to 72 mg of myristyl amine and heated with constant mixing until a uniform paste was formed. Glutamic acid 65 mg in 5 ml of distilled deionized water was added to the paste and heated until a gel was formed. Phosphate buffered saline was added to the gel to form liposomes.

30

Example #11DLPC Containing Liposomes

Dilaurylphosphatidylcholine (DLPC) 50 mg was mixed with 20 mg oleic acid to form a homogeneous paste. Arginine 20 mg was added to 10 ml of phosphate buffered saline, added to the paste and hand mixed until a turbid liposome solution formed.

Example # 12Phosphatidylethanolamine-Glutamic Acid Liposomes

1 L-glutamic acid 32 mg was dissolved in 2.0 ml of
distilled deionized water and the pH adjusted to 5.2 with
5 1.0 N sodium hydroxide. This solution was heated to
60°C, and 100 mg of phosphatidylethanolamine added. The
solution was kept at 60°C with constant mixing until a
uniform viscous gel was observed.

The phosphatidylethanolamine-glutamic acid gel was
10 diluted 1/10 by phosphate buffered saline. Vesicular like
structures are observed under phase contrast light
microscopy.

Example #13Dipivalylepinephrine Liposomes

15 One gram of dipalmitoylphosphatidylcholine was mixed
with 396 mg of oleic acid, until a homogeneous paste
formed. Then 400 mg of arginine in 20 ml of distilled
deionized water was added to form a pre-vesicle clear gel.

20 To make the liposomes, 242 mg of dipivalylepinephrine
was dissolved in 10 ml of distilled deionized water. Then
5 grams of the pre-vesicle gel was mixed with 5 grams of
the dipivalylepinephrine solution after which 50 ml of
phosphate buffered saline was added forming a liposome
25 solution.

Example #14Flurbiprofen Liposomes

To make these liposomes, 980 mg of
30 dipalmitoylphosphatidylcholine, 370 mg of oleic acid, and
320 mg of flurbiprofen (free acid) were mixed together
until a homogeneous paste was observed. Then 510 mg of
arginine in 10 ml of purified water was added to the paste
and heated to 41°C with constant mixing for 30 minutes.

35 A clear pre-vesicle gel formed of which 5 grams was
introduced into 50 ml of phosphate buffered saline and

- 1 mixed with a stir bar until a bluish translucent solution was observed.

Example #15

5 Levobunolol Liposomes

Thirty mg of dipalmitoylphosphatidylcholine and 15 mg of cholesterol were weighed into a 4 ml vial. Ten mg of linoleic acid was added and mixed together to form a uniform paste. Two ml of a 1% aqueous levobunolol solution containing 10 mg of arginine was added to the paste and mixed together. Then 10 ml of phosphate buffer solution was added and heated to 45°C to form liposomes.

Example #16

15 Pilocarpine Liposomes

To 120 mg of dipalmitoylphosphatidylcholine was added 40 mg of oleic acid to form a homogeneous paste. Forty mg of pilocarpine free base was added to 10 ml of distilled deionized water. This solution was added to the paste and heated to 45°C to form a pre-liposome gel. The resulting gel was diluted with 20 ml of phosphate buffered saline to form liposomes.

Example #17

25 Epinephrine Liposomes

Dipalmitoylphosphatidylcholine 250 mg was mixed with 100 mg of oleic acid to form a homogeneous paste. 50 mg of epinephrine, free base, was dissolved in 5.0 ml of distilled deionized water, heated to 40°C and added to the dipalmitoylphosphatidylcholine/oleic acid paste. This solution was mixed until a homogeneous viscous creamy gel was observed. This gel was diluted 1/5 with phosphate buffered saline (pH 7.22) to form liposomes.

35

-20-

EXAMPLE #18Effect of Arginine Concentration on Liposome Size

To 502 mg of dipalmitoylphosphatidylcholine (DPPC) was added 10 microliters of (2-palmitoyl-1-C¹⁴) (0.1 mCi/ml) dipalmitoylphosphatidylcholine. Chloroform was added to effect complete mixing of the radioactivity and then evaporated. Oleic acid (OA), 195 mg, was then mixed into the lipid to form a paste. Five ml of distilled water containing 119 mg of arginine was added and mixed at 45°C to form a clear gel.

One gram of the gel was weighed into four different vials and arginine was added as follows:

15	Sample ID	Sample Composition DPPC:OA:Arg
	Vial 1 + 1 ml water	(1:1:1)
	Vial 2 + 1 ml of 50 mg/ml Arg in H ₂ O	(1:1:3)
20	Vial 3 + 1 ml of 84 mg/ml Arg in H ₂ O	(1:1:5)
	Vial 4 + 1 ml of 192 mg/ml Arg in H ₂ O	(1:1:10)

25

One-half gram of each solution was diluted in 50 ml of phosphate buffered saline of pH 7.8.

The estimated weight diameter was obtained from a Sephracyl S-1000 column chromatographic analysis employing ¹⁴C-isotope labelled DPPC. The effects are given in the following Table.

35

1

Table IIEffects of Arginine Concentration on Vesicle Size

	<u>System</u>	<u>pH</u>	<u>Estimated Weight Diameter (nm)</u>
5	DPPC:OA:Arg (1 :1: 1)	7.8	~220
	DPPC:OA:Arg (1 :1: 3)	7.8	~140
	DPPC:OA:Arg (1 :1: 5)	7.8	~90
10	DPPC:OA:Arg (1 :1: 10)	7.8	~20

EXAMPLE #19

15

pH Effect on Vesicle Size

Additionally, the vesicle size can be varied by varying the pH of the aqueous buffer solution.

To 100 mg of dipalmitoylphosphatidylcholine (DPPC) was added 25 microliters of (2-palmitoyl-1-C¹⁴) (0.1 20 mCi/ml) dipalmitoylphosphatidylcholine. Chloroform was added to effect complete mixing of the radioactivity and then evaporated. Oleic acid (OA), 40.1 mg, was then mixed into the lipid to form a paste. One ml of a solution containing 24 mg/ml arginine in water was added to the 25 lipid mixture and mixed at 45°C to form a clear gel.

Two 100 mg aliquots of this gel were diluted in 10 ml of phosphate buffer at pH 9.0 and 7.4 respectively.

Again, the estimated weight diameter (A) was obtained from the Sephracryl S-1000 column chromatographic analysis 30 employing ¹⁴C-isotope labelled dipalmitoylphosphatidylcholine. Results are given in the following Table.

35

16501

Table III
pH Effects on Vesicle Size

	System	Estimated Weight	
		pH	Diameter (nm)
5	DPPC:OA:Arg (1 :1: 1)	7.4	~>300
	DPPC:OA:Arg (1 :1: 1)	7.8	~220
10	DPPC:OA:Arg (1: 1 :1)	9.0	~25.4

Thus, a desired size of the liposomal vesicles can be prepared by varying the arginine concentration or the pH of the aqueous buffer solution.

EXAMPLE #20

Liposome Stability

Sterile liposomes may be prepared from the heat sterilized pre-liposome gel. Alternatively, the liposome gel or the liposomes may be sterile filtered through an appropriate sterilizing filter.

Liposomes prepared from DPPC:OA:Arg (1:1:2) at pH 8.0 were heat sterilized and stored at room temperature for approximately one year without adding antimicrobial agents and anti-oxidants. No bacterial growth, discoloration and precipitation were observed. Negative stain electron microscopic examination of the one year old liposomes revealed that the liposomal vesicles are stable.

EXAMPLE #21

Encapsulated sucrose latency was measured using C^{14} -sucrose encapsulated with the DPPC:OA:Arg (1:1:1) liposome system in aqueous phosphate buffer solution at pH 7.8. The result was presented in Table IV.

Table IV

<u>Days</u>	<u>% Sucrose Latency</u> <u>% Latency</u>
0	100
1	97.4
3	93.4
7	91.4

Thus, the present liposome system has an excellent latency for drug delivery.

EXAMPLE #22Efficiency of Encapsulation

A number of drugs were encapsulated with 10 mg/ml DPPC:oleic acid:Arg (1:1:1) liposomes to illustrate medicament encapsulation for use as a drug delivery system. The results are presented in Table V.

Table V

<u>Drugs</u>	<u>Entrapment of Drugs</u> <u>pH</u>	<u>% Entrapment</u>
Flurbiprofen	7.8 PBS	90%
Dipivalyl Epinephrine	7.1 PBS	80%

Example #23Lyophilized Liposomes

Oleic acid, 30.0 gm, and 7.5 gm of cholesterol U.S.P. were confectioned. Then 75.0 gm of phosphatide type 20 powder (Asahi Chemical Co.) was mixed with the oleic acid/cholesterol mixture until an homogeneous paste was formed.

Then 15.0 gm of arginine (free base) was dissolved in 183 gm of distilled, deionized water. This arginine solution was mixed slowly with the lipid paste to form a homogeneous gel. The gel pH was adjusted to 7.4 using 5.0

1 N HCl.

A 10.0 gm aliquot of this pre-liposome gel was transferred to a 10 ml vial and lyophilized. The resulting powder formed liposomes when diluted with 5 ml of phosphate buffered saline.

EXAMPLE #24

Acid Stable Liposome Preparations

An example of acid stable liposomes employing this invention is illustrated by liposomes prepared with the following materials: distearoylphosphatidylcholine dipalmitoyl phosphatidylcholine: oleic acid, arginine, and cholesterol. These materials were combined in a molar ratio of 1:2:2:2:0.2 as follows: Cholesterol, 20 mg., was mixed with 144 mg. of oleic acid and treated to 40°C. DSPC, 200 mg., and 400 mg. of DPPC was added and mixed at 40°C. The mixture was stirred until a uniform homogeneous paste was formed.

Arginine, 88 mg., was dissolved in 1.15 g. of deionized distilled water. This arginine solution was added to the lipid paste and mixed at about 45°C until a homogeneous pre-liposome gel formed. The pH of the gel was adjusted to various pH levels with 0.1N HCl. The gel was diluted 10-fold with 0.9% NaCl, forming vesicles.

Liposome Stability at pH 4.4

<u>Time/Days</u>	<u>Size/(NMx10³)</u>
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0	1.024
3	1.136
7	1.127

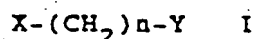
1 CLAIMS (BE, CH, DE, FR, GB, IT, LI, LU, NL and SE)

1. A liposome composition made by dispersing in an aqueous medium in a manner adequate to form liposomes:

- 5 a. liposome-forming material;
 b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material; and
 c. optionally material to be encapsulated.

10

2. The composition of claim 1 wherein the hydrating agent is an alpha amino acid having an omega substitution which is a carboxylate, amino, guanidino function or a pharmaceutically acceptable salt thereof or a compound of
 15 the formula:



wherein

- X is $H_2N-C(NH)-NH-$, H_2N- , ZO_3S- , Z_2O_3P- , or ZO_2C- wherein Z is H or an inorganic or organic cation;
 20 Y is $-CH(NH_2)-CO_2H$, $-NH_2$, $-NH-C(NH)-NH_2$, $-COOH$, $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

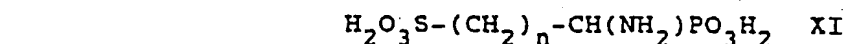
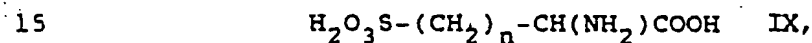
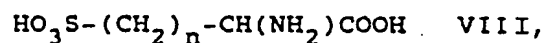
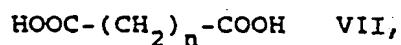
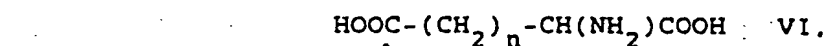
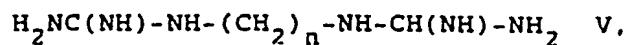
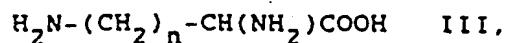
25

3. The composition of claim 2 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

30

4. The composition of claim 3 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:

35



wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

5. The product of claim 4 wherein said hydrating
25 agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

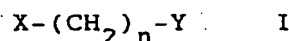
6. The composition of claim 4 wherein said hydrating
30 agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

7. A composition capable of forming liposomes when dispersed in an aqueous medium and the liposomes made
35 therefrom, which composition comprises:

a. liposome-forming material;

- 1 b. a hydrating agent wherein the hydrating agent is
 present in a molar ratio of between 1:20 and 1:0.05
 relative to the liposome-forming material;
 c. optionally material to be encapsulated; and
 5 d. water in an amount up to 300 moles relative to
 the solids.

8. The composition of claim 7 wherein the
 hydrating agent is an alpha amino acid having an omega
10 substitution which is a carboxylate, amino, or guanidino
 function or a pharmaceutically acceptable salt thereof,
 or a compound of the formula:

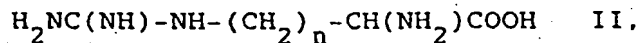


wherein

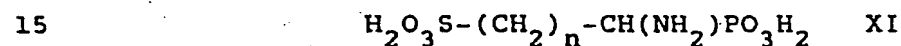
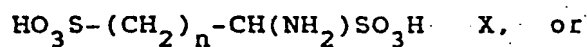
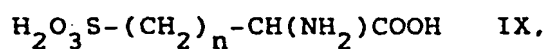
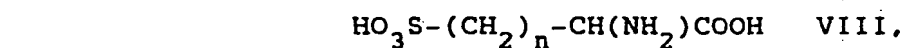
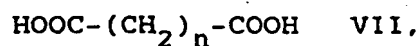
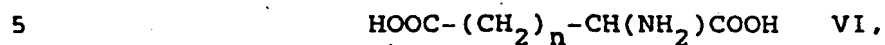
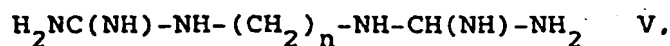
- 15 X is $H_2N-C(NH)-NH-$, H_2N- , ZO_3S- , Z_2O_3P- , or
 ZO_2C- wherein Z is H or an inorganic or organic cation;
 Y is $-CH(NH_2)-CO_2H$, $-NH_2$, $-NH-C(NH)-NH_2$, $-COOH$,
 $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined
 above; and
20 n is the integer 1-10; or
 a pharmaceutically acceptable salt thereof.

9. The composition of claim 8 wherein the hydrating
 agent is present in an amount between 1:2 to 1:0.5 molar
25 ratio relative to the liposome-forming material.

10. The product of claim 9 wherein said hydrating
 agent is arginine, homoarginine, or their N-acyl
 derivatives, gamma-aminobutyric acid, asparagine, lysine,
30 ornithine, glutamic acid, aspartic acid or a compound of
 the formulae:



- 35 $H_2N-(CH_2)_n-CH(NH_2)COOH \quad III.$



wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

20 11. The composition of claim 10 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

25 12. The composition of claim 10 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

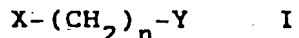
30 13. A means for forming stable liposomes wherein the means comprises adding to liposome-forming materials before or at the time they are dispersed in an aqueous medium, a hydrating agent in a molar ratio of 1:20 to 1:0.05 relative to the liposome-forming material.

1 14. A substantially dry composition capable of
forming liposomes when dispersed in an aqueous medium
wherein the composition comprises:

- (a) a liposome forming material;
- 5 (b) a hydrating agent where the hydrating agent is
present in a molar ratio between 1:20 and 1:0.05
relative to the liposome forming material; and
- (c) optionally material to be encapsulated.

10 which composition is prepared by mixing in an aqueous
medium the materials of (a), (b) and (c), and dehydrating
said mixture to give said substantially dry composition.

15 15. The composition of claim 14 wherein the
hydrating agent is an alpha amino acid having an omega
substitution which is a carboxylate, amino, or guanidino
function or a pharmaceutically acceptable salt thereof,
or a compound of the formula:



wherein

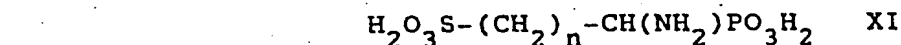
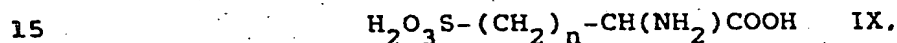
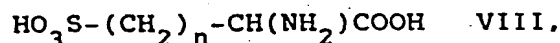
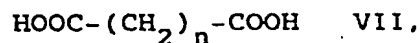
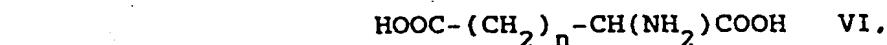
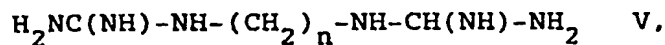
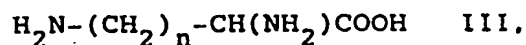
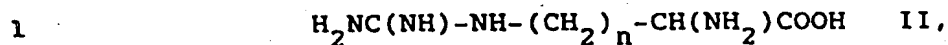
20 X is $H_2N-C(NH)-NH-$, H_2N- , ZO_3S- , Z_2O_3P- , or
 ZO_2C- wherein Z is H or an inorganic or organic cation;

Y is $-CH(NH_2)-CO_2H$, $-NH_2$, $-NH-C(NH)-NH_2$, $-COOH$,
 $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined
above; and

25 n is the integer 1-10; or
a pharmaceutically acceptable salt thereof.

30 16. The composition of claim 15 wherein the
hydrating agent is present in an amount between 1:2 to
1:0.5 molar ratio relative to the liposome-forming
material.

35 17. The product of claim 16 wherein said hydrating
agent is arginine, homoarginine, or their N-acyl
derivatives, gamma-aminobutyric acid, asparagine, lysine,
ornithine, glutamic acid, aspartic acid or a compound of
the formulae:



wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

25 18. The composition of claim 17 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

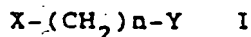
30 19. The composition of claim 17 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

CLAIMS (AT)

1. A method for making a liposome composition which method comprises dispersing in an aqueous solution in a manner adequate to form liposomes:

- a. liposome-forming material;
- b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material; and
- c. optionally material to be encapsulated.

2. The method of claim 1 wherein the hydrating agent is an alpha amino acid having an omega substitution which is a carboxylate, amino, guanidino function or a pharmaceutically acceptable salt thereof or a compound of the formula



wherein

X is $H_2N-C(NH)-NH-$, H_2N- , ZO_3S- , Z_2O_3P- , or ZO_2C- wherein Z is H or an inorganic or organic cation;

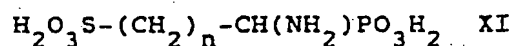
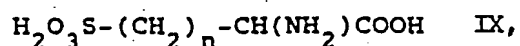
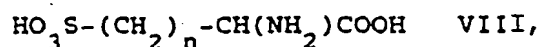
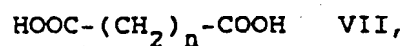
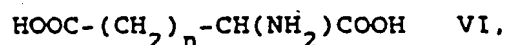
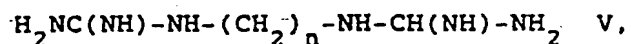
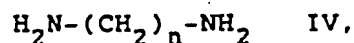
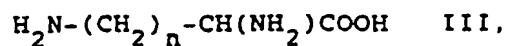
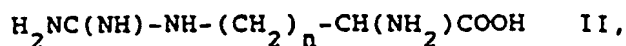
Y is $-CH(NH_2)-CO_2H$, $-NH_2$, $-NH-C(NH)-NH_2$, $-COOH$, $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined above; and

n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

3. The method of claim 2 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

4. The method of claim 3 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:



wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

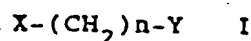
5. The method of claim 4 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

6. The method of claim 5 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

7. A method for making a liposome composition which method comprises dispersing in an aqueous solution in a manner adequate to form liposomes, a composition comprised of:

- a. liposome-forming material;
- b. a hydrating agent wherein the hydrating agent is present in a molar ratio of between 1:20 and 1:0.05 relative to the liposome-forming material; and
- c. water in an amount up to 300 moles relative to the solids; and
- d. optionally, material to be encapsulated.

8. The method of claim 7 wherein the hydrating agent is an alpha amino acid having an omega substitution which is a carboxylate, amino, or guanidino function or a pharmaceutically acceptable salt thereof or a compound of the formula



wherein

X is $H_2N-C(NH)-NH-$, H_2N- , ZO_3S- , Z_2O_3P- , or ZO_2C- wherein Z is H or an inorganic or organic cation;

Y is $-CH(NH_2)-CO_2H$, $-NH_2$, $-NH-C(NH)-NH_2$, $-COOH$, $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined above; and

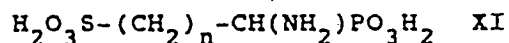
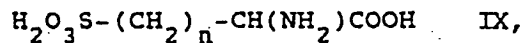
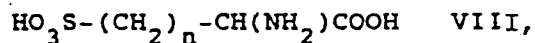
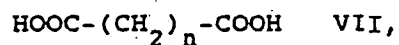
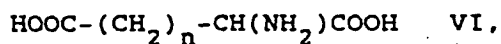
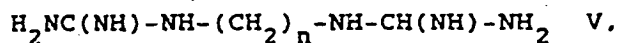
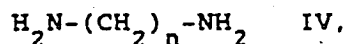
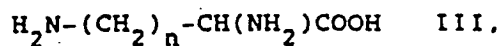
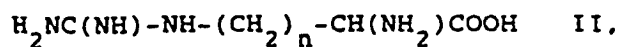
n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

9. The method of claim 8 wherein the hydrating agent is present in an amount between 1:2 to 1:0.5 molar ratio relative to the liposome-forming material.

10. The method of claim 9 wherein said hydrating agent is arginine, homoarginine, or their N-acyl derivatives, gamma-aminobutyric acid, asparagine, lysine, ornithine, glutamic acid, aspartic acid or a compound of the formulae:

-34-



wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

11. The method of claim 10 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

12. The method of claim 10 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.

1 13. A method of preparing a substantially dry
composition capable of forming liposomes when dispersed
in an aqueous medium wherein the composition comprises:

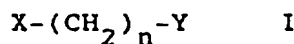
(a) a liposome forming material;

5 (b) a hydrating agent where the hydrating agent is
present in a molar ratio between 1:20 and 1:0.05
relative to the liposome forming material; and

(c) optionally material to be encapsulated,

10 which method comprises mixing in an aqueous medium the
materials of (a), (b) and (c), and dehydrating said
mixture to give said substantially dry composition.

14. The method of claim 13 wherein the hydrating
agent is an alpha amino acid having an omega substitution
15 which is a carboxylate, amino, or guanidino function or a
pharmaceutically acceptable salt thereof, or a compound
of the formula:



wherein

20 X is $H_2N-C(NH)-NH-$, H_2N- , ZO_3S- , Z_2O_3P- , or
 ZO_2C- wherein Z is H or an inorganic or organic cation;

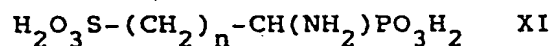
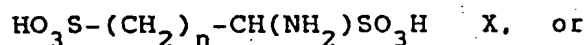
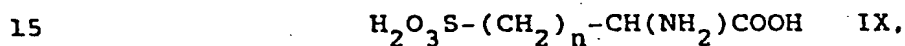
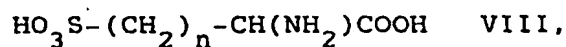
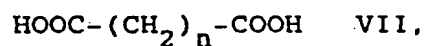
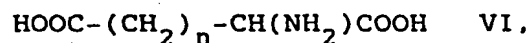
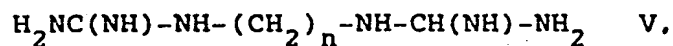
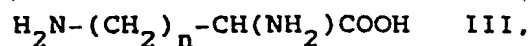
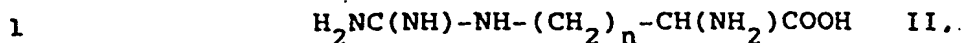
Y is $-CH(NH_2)-CO_2H$, $-NH_2$, $-NH-C(NH)-NH_2$, $-COOH$,
 $CH(NH_2)SO_3Z$ or $ZH(NH_2)PO_3Z_2$ wherein Z is defined
above; and

25 n is the integer 1-10; or

a pharmaceutically acceptable salt thereof.

15. The method of claim 14 wherein the hydrating
agent is present in an amount between 1:2 to 1:0.5 molar
30 ratio relative to the liposome-forming material.

16. The method of claim 15 wherein said hydrating
agent is arginine, homoarginine, or their N-acyl
derivatives, gamma-aminobutyric acid, asparagine, lysine,
35 ornithine, glutamic acid, aspartic acid or a compound of
the formulae:



wherein n is 2-4, or a pharmaceutically acceptable salt thereof.

25 17. The method of claim 16 wherein said hydrating agent is arginine, homoarginine, gamma-aminobutyric acid, lysine, or ornithine or a pharmaceutically acceptable salt thereof.

30 18. The method of claim 16 wherein said hydrating agent is glutamic acid or aspartic acid or a pharmaceutically acceptable salt thereof.



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0211647

Application number

DOCUMENTS CONSIDERED TO BE RELEVANT			EP 86306014.1
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A	DE - A1 - 2 706 705 (TAKEDA CHEMICAL INDUSTRIES) * Claims 1,17-19,26; page 11, lines 16-17; page 12, lines 29-31; examples 39,40 * --	1-5,7-11,13-18	A 61 K 9/50 A 61 K 9/52 A 61 K 9/10 A 61 K 47/00 B 01 J 13/02
A	EP - A1 - 0 120 722 (PARFUMS CHRISTIAN DIOR) * Examples 7,8,9 * --	1-4,7-10,13	/A 61 K 31/195
X	EP - A1 - 0 087 993 (PARFUMS CHRISTIAN DIOR) * Claims 1,7,8; examples 11,12; page 17, lines 4-13 * --	1-4,6-10,12,13	
X	EP - A2 - 0 092 453 (THE LIPOSOME CORPORATION) * Abstract; claims 1,10,14,16, 19,28,29,31,32; page 11, line 23 - page 14, line 18; page 35, line 32 - page 36, line 12; sections 6.1-6.3 * --	1,7,13	TECHNICAL FIELDS SEARCHED (Int. Cl.4) A 61 K 9/00 B 01 J 13/00
X	US - A - 3 957 971 (W.S.OLEUIACZ) * Abstract; claims 1,11,12; column 5, lines 18-45 * --	1-4,6-13	
X	US - A - 3 932 657 (Y.E.RAHMANN) * Abstract; column 3, line 44 - column 4, line 33 * --	1,7,13	
The present search report has been drawn up for all claims			
Place of search VIENNA		Date of completion of the search 14-11-1986	Examiner MAZZUCCO
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	



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Application number

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EP 86306014.1

DOCUMENTS CONSIDERED TO BE RELEVANT				
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)	
X	WO - A1 - 84/02 076 (FLUID-CARBON INTERNATIONAL AB) * Abstract; claims 1,2,4,19,26-28,32; examples 4,6 *	1,7,13		
X	GB - A - 2 047 535 (A.NATTERMANN & CIE GMBH) * Claims 1,3,5; examples 1,2 *	1,7,13 14		
X	GB - A - 2 041 871 (FARMITALIA CARLO ERBA S.P.A.) * Abstract; claims 7,8; examples 1,2 *	1,7,13 14		
X	DE - A1 - 2 856 333 (A.NATTERMANN & CIE GMBH) * Claims 1-4,7-9; examples 2,4 *	1-5,7- 11,13- 18		TECHNICAL FIELDS SEARCHED (Int. Cl.4)
A	AT - B - 356 278 (F.HOFFMANN - LA ROCHE & CO AKTIENGESellschaft) * Claim 1 *	1,2,7, 8,13		
A	DE - A1 - 2 656 333 (K.THEURER) * Example 2 *	1,2,4, 5,7,8, 10,11, 13		
A	CH - A - 498 627 (K.LARSSON) * Claims 1,2; column 3, lines 22-31,60-67; column 4, lines 22-26 *	1,7,13		
The present search report has been drawn up for all claims				
Place of search VIENNA		Date of completion of the search 14-11-1986	Examiner MAZZUCCO	
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>				



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Application number

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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	US - A - 4 174 296 (G.S.KASS) * Claim 1 *	1	
A	EP - A2 - 0 130 577 (DAIICHI SEIYOKU CO. LTD.) * Pages 4,5,8; abstract; claims 1,4-7,11-13 *	1,7,13	
A	EP - A2 - 0 102 324 (CIBA-GEIGY AG) * Abstract; claims 1,17; page 23, lines 18-23; page 24, line 22 - page 25, line 15 *	1,2,7,8,13,14	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
The present search report has been drawn up for all claims			
Place of search VIENNA		Date of completion of the search 14-11-1986	Examiner MAZZUCCO
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>			